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(54) **Bacterial beta-galactosidase, immobilized forms thereof, process for their preparation and use in the hydrolysis of lactose, and novel bacteria.**

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Bacterial beta-galactosidase, immobilized forms thereof, process for their preparation and use in the hydrolysis of lactose, and novel bacteria.

This invention relates to enzymes and processes utilising enzymes, in particular to β-galactosidase enzymes derived from Bacillus stearothermophilus and their use in the enzymatic hydrolysis of lactose.

Lactose is the sugar component of whey, the liquid which remains after the separation of solids from milk or cream during customary cheese-making processes, and which at the present time is often regarded as a waste material and presents a disposal problem. In view of its low sweetness and solubility and tendency to undesirable crystallisation, lactose is unsatisfactory for use as a food sugar. Lactose, however, is a dimer of glucose and galactose and may be hydrolysed to its separate sugar units which are then readily utilisable as food sugars. Straightforward acid hydrolysis of lactose may be used, though this is not usually satisfactory because side reactions take place giving rise to a product having undesirable flavours. Alternatively hydrolysis may be effected by use of a β-galactosidase enzyme, although the temperature optimums for known β-galactosidase enzymes, i.e. from about 30—40°C up to a maximum of about 50°C, permit microbial growth which seriously contaminates the product.

New β-galactosidase enzymes have now been discovered which may be used for hydrolysis of lactose whilst avoiding the problem of contamination by adventitious microbial growth.

Accordingly, the present invention provides a thermally stable β-galactosidase enzyme, derived from a strain of *Bacillus stearothermophilus*, characterised in that it has a thermal stability such that when in purified form it has an activity half life of at least 1½ hours at 55°C, at least 1 hour at 60°C and at least 10 minutes at 65°C, as measured using ONPG as substrate, and in that it is derived from a strain of *Bacillus stearothermophilus* identified as NCIB 11407, NCIB 11408, NCIB 11409, NCIB 11410, NCIB 11411, NCIB 11412 and NCIB 11413 or a variant or mutant thereof. The invention also includes compositions of immobilised enzyme and enzymically active whole cell preparations of bacteria of species *B. stearothermophilus* which contain this enzyme.

A useful indicator of the suitability of strains of *Bacillus stearothermophilus* for derivation of the β-galactosidase of the invention is ability to grow at elevated temperatures, usually at least 60° and preferably at least 65°C, utilising lactose as sole carbon source. A basal salts culture medium containing lactose may be used for growth of the bacterium e.g. a basal salts medium as hereinafter specifically described, and evidence of growth may be determined by methods well known in the art. Growth at elevated temperatures is only an indicator, however, and suitability is ultimately determined by the thermal stability and persistence of the β-galactosidase derived from the strain of *B. stearothermophilus* in question. For the purpose of the present description, the thermal stability of the enzyme is defined in terms of its activity half life, with respect to ONPG (orthonitrophenyl-β-D-galactopyranoside) as substrate, at various temperatures. Preferably, the thermal stability of the enzyme is such that when in purified form it has an activity half life of at least 5 hours at 55°C, at least 3 hours at 60°C, at least 1 hour at 65°C, and especially also at least ten minutes at 70°C. The activity half lives given above are typically measured using purified enzyme extract. Whole cell preparations may exhibit slightly longer half life periods than purified enzyme extracts at the higher temperatures i.e. 65 and 70°C, used and typical activity half lives for whole cell preparations according to the invention are at least 45 minutes at 65°C and at least 5 minutes at 70°C.

Cultures of strains of *B. stearothermophilus* useful in the present invention were deposited with the National Collection of Industrial Bacteria, Torry Research Station, Aberdeen, Scotland on the 24th of May 1978, and the deposits are identified by the reference numbers NCIB 11407, NCIB 11408, NCIB 11409, NCIB 11410, NCIB 11411, NCIB 11412 and NCIB 11413, which are hereinafter referred to as the HRI A/S 1, A/S 2, A/S 3, A/S 4, A/S 5, A/S 6 and A/S 8 strains respectively of *B. stearothermophilus*. The useful strains also include variants and mutants of the HRI strains of *B. stearothermophilus* and such variants and mutants are new, and together with the HRI strains are included within the scope of the invention. The HRI strains have been derived from continuous cultures grown over a considerable period of time, preferably using lactose as sole carbon source, and it is believed that the HRI strains may have arisen as mutants and been preferentially selected during this culturing.

The HRI organisms have been identified as strains of *B. stearothermophilus* from the following characteristics: they are non-motile, rod-shaped (typically 0.6 μ wide, 2.0 μ long, from scanning electron micrographs), have a positive Gram stain and form spores. They are obligate aerobes, all hydrolyse urea, all but one hydrolyse casein and many also hydrolyse starch. All produce acid from lactose, mannitol, sucrose and maltose, and all but one are insensitive to azide. The organisms do not appear to grow at 30 or 37°C, and have a minimum growth temperature of about 45°C and a maximum growth temperature of about 65°C. Advantageously, also, the HRI strains of *B. stearothermophilus* are typically constitutive for β-galactosidase production, i.e. give rise to the β-galactosidase of the invention independent of the substrate on which the organisms are grown.

The characteristics of the various HRI strains of *B. stearothermophilus* given below in Table 1 characterise these organisms from other strains of *B. stearothermophilus*. It will be appreciated,

however, that other strains of *B. stearothermophilus*, having the same or similar characteristics may be suitable for derivation of the novel β-galactosidase of the invention.

TABLE 1

Characteristics of HR1 strains of B. stearothermophilus

Strain	HRI A/S 1	HRI A/S 2	HRI A/S 3	HRI A/S 4	HRI A/S 5	HRI A/S 6	HRI A/S 8
Gram stain	+	+	+	+	+	+	+
Spores	√	√	√	√	√	√	√
Motility	–	–	–	–	–	–	–
Catalase production	+	+	+	+	+	+	+
Temperature max. 65°	+	+	+	+	+	+	+
min. 45°	+	+	+	+	+	+	+
30°	–	–	–	–	–	–	–
Acid production from ARABINOSE	+	+	–	–	+	+	+
XYLOSE	+	+	–	–	+	+	+
MANNITOL	+	+	+	+	+	+	+
SUCROSE	+	+	+	+	+	+	+
LACTOSE	+	+	+	+	+	+	+
GLUCOSE	+	+	+	+	+	+	+

MALTOSE	+	+	+	+	+	+	+
Voges-Proskauer	–	–	–	–	–	–	–
Growth on:–							
5% NaCl	+	+	+	+	+	+	–
0.02% Azide	–	+	+	+	+	+	+
0.001% Lysozyme	+	–	+	+	+	+	+
Decomposition of:–							
Casein	+	+	–	+	+	+	+
Starch	+	+	–	–	–	+	+
Urea	+	+	+	+	+	+	+
Specific Activity of β-galactosidase from cells grown on nutrient broth (μ mole/min/mg protein) at 65°C	0.09	0.36	0.10	0.05	0.35	4.61	4.07
All incubations done at 65°C except where stated otherwise							

The novel β-galactosidase enzyme system of the invention, as derived from the HRI strains of *B. stearothermophilus* and variants and mutants thereof (those derived from the HRI strains of *B. stearothermophilus* being hereinafter termed the HRI enzyme), are typically distinguished from other β-galactosidases derived from prior art strains of *B. stearothermophilus*, such as the strain described by Goodman and Pedersen (Canadian Journal of Microbiology, 22 (1976), 817—825) by the following characteristics:

1. The novel enzymes characteristically have significantly greater thermal stability. For instance, the purified HRI enzyme shows no change in activity after 90 minutes incubation at 55°C, an activity half life of 450 minutes after incubation at 60°C, an activity half life of 85 minutes after incubation at 65°C, and an activity half life of 20 minutes after incubation at 70°C, as assayed using ONPG as substrate at pH 7.0.

2. The novel enzymes typically have significantly greater affinity for substrates e.g. lactose and ONPG, than prior art enzymes. For example, the HRI enzyme has Michaelis constants (Km) of 0.43 mM and 2.1 mM for substrates ONPG and lactose respectively, as measured at 65°C.

3. The novel enzymes are typically activated by magnesium ion; the HRI enzyme being activated by 60% on addition of magnesium ion at a level of 10 mM.

4. The novel enzymes are generally significantly more active at acid pHs than prior art β-galactosidases. For example, the purified HRI enzyme has an activity maximum of a 100% at pH 6.0 and activities ranging from about 10% at pH 5 to 53% at pH 7.5.

These and various other characteristics will be hereinafter described in more detail with reference to the specific case of the HRI enzyme; although, without prejudice to the scope of the invention, it is believed that in general the enzymes of the invention will exhibit similar characteristics.

The enzyme, enzyme compositions and enzymatically active whole cell preparations of the invention are derived by first cultivating a culture of a suitable strain of *B. stearothermophilus*. Cultivation is typically carried out at elevated temperature, generally at least 60°C, preferably about 65°C, on a suitable culture medium, such as a basal salts culture medium, comprising lactose as sole carbon source. Any suitable cultivation regime may be employed including either batch or continuous culture. The purified enzyme or partially purified enzyme extracts and compositions comprising the enzyme are obtained by extraction of enzyme from the cells e.g. by lysing, followed by purification procedures as required. Purified enzyme or enzyme extracts may be immobilised on or with a suitable solid phase material such as an ion-exchange material e.g. DEAE-cellulose or like material, to provide preferred immobilised enzyme composition products.

Whole cell preparations may be subjected to preparative treatment to render the cells permeable, and thus enhance apparent enzyme activity for lactose hydrolysis. One treatment which may be used to

render cells permeable is treatment with toluene, e.g. toluene — acetone solution. Permeable whole cell preparations may preferably be immobilised, for instance, in a gel matrix to provide an advantageously immobilised enzymatically active whole cell preparation. In particularly preferred embodiments, however, permeable whole cell preparations are immobilised with a suitable ion-exchange support material, including in particular DEAE-cellulose and like ion-exchange materials such as amino ethyl cellulose, DEAE-Sephadex and DEAE-Sepharose.

Preferred processes for production of immobilised enzyme composition products or especially immobilised whole cell products, immobilised with ion-exchange support materials, comprise prior treatment of the ion exchange material with glutaraldehyde or a similar linking reagent e.g. carbodiimides so as to covalently attach the enzyme or cells to the ion exchange support material. Such prior treatment, in particular with DEAE-cellulose and like ion-exchange support materials, advantageously gives products having greater retention of enzyme activity than products prepared without such prior treatment, and preferably also products of outstanding thermal stability. For example, whole cell products immobilised on DEAE-cellulose which has been pre-treated with glutaraldehyde often have activity half lives of at least 7 days, in some cases about 15 days, at 60°C and pH 7.

The products of the invention may be used in processes for the hydrolysis of lactose to glucose and galactose.

Thus in a further aspect the invention includes a process for the hydrolysis of lactose to a product comprising glucose and galactose, in which lactose is contacted with a novel β-galactosidase enzyme, composition or enzymatically active whole cell preparation according to the invention. Typically the process is carried out at elevated temperature, usually at a temperature of at least 55°C, preferably at a temperature of at least 60°C, especially about 65°C. Generally the procedures and apparatus employed for hydrolysis of lactose are similar to those commonly used in equivalent or other enzymatic hydrolysis processes and a wide range of possible systems and procedures will be apparent to workers skilled in the art. Thus in a preferred embodiment, in a continuous incubation mode, substrate containing lactose in solution form is passed into a reactor containing the enzyme or enzymatically active whole cell preparation, preferably in immobilised form e.g. immobilised enzyme composition or immobilised whole cell preparation, and the glucose and galactose products are recovered downstream of the reactor.

Any suitable source of lactose may be used as the substrate in the process of the invention, for instance the lactose may be derived by purification from whey or other suitable source. Preferably, however, the crude whey itself or other lactose containing milk product is contacted directly with the enzyme, composition or enzymically active whole cell preparation, e.g. in a reactor. In this latter respect the use of the enzyme of the invention is particularly advantageous in view of the magnesium ion activation which it exhibits and the advantageous breadth of pH compatibility of the enzyme. Generally also, however, the use of the process of the present invention is particularly advantageous in that the elevated temperatures used, e.g. at least 55°C and preferably at least 60°C, significantly diminish the risk of contamination by adventitious microbial growth. Furthermore, the strains of *B. stearothermophilus* used do not appear to grow at 37°C and thus do not present a substantial contamination problem for the glucose/galactose product.

Moreover, in view of the desirable high thermal stability of the enzyme of the invention, the enzyme may be incorporated directly with UHT milk products prior to heat treatment and may be advantageously sterilised along with the milk during heat treatment and subsequently convert lactose contained therein to glucose and galactose.

The process of the invention provides a lactose hydrolysate product comprising glucose and galactose, and may be in partially or fully hydrolysed form. The hydrolysate may be concentrated to produce hydrolysate syrups which may find advantageous uses in the confectionery and brewing industries. Alternatively the glucose and/or galactose produced may be recovered in purified form and further utilised as required.

The invention is further described by way of illustration only in the following examples which refer to the accompanying diagrams, in which;

Figure 1 is a graph of β-galactosidase activity of purified enzyme, whole cells and immobilised whole cells at various temperatures;

Figure 2 is a similar graph of β-galactosidase activity at various pHs;

Figure 3 is a graph showing the β-galactosidase activities of purified enzyme, whole cells and immobilised whole cells for lactose hydrolysis in demineralised whey;

Figure 4 is a graph showing the β-galactosidase activity of purified enzyme for the hydrolysis of lactose in whole milk;

Figure 5 is a graph showing the β-galactosidase activity of cells immobilised on various cellulosic ion-exchange support materials;

Figure 6 is a graph of β-galactosidase activity of DEAE-cellulose immobilised cells against substrate flow rate;

Figure 7 is a graph showing the β-galactosidase activity of DEAE-cellulose immobilised cells against pH;

Figure 8 are graphs showing β-galactosidase activity of DEAE-cellulose immobilised cells against temperature;

Figure 9 is a graph showing β-galactosidase activity of DEAE-cellulose immobilised cells against ionic strength; and

Figure 10 is a graph showing β-galactosidase activity of DEAE-cellulose immobilised cells for various lactose substrates.

Example 1

Growth of bacterium *Bacillus stearothermophilus*

The basal salts medium used for batch and continuous culture of strains of *B. stearothermophilus* is as follows:—

nitrilotriacetic acid	100 mg/l
$MgSO_4 7H_2O$	100 mg/l
$CaSO_4 2H_2O$	60 mg/l
NaCl	8 mg/l
KNO_3	103 mg/l
$NaNO_3$	689 mg/l
Na_2HPO_4	111 mg/l
Conc: H_2SO_4	0.5 ml/l
$FeCl_3$	0.28 mg/l
$MnSO_4H_2O$	2.2 mg/l
$ZnSO_4$	0.5 mg/l
H_2BO_3	0.5 mg/l
$CuSO_4$	0.016 mg/l
$Na_2MoO_4 \cdot 2H_2O$	0.025 mg/l
$CaCl_2 6H_2O$	0.046 mg/l

Lactose is added to this basal salts culture medium, as sole carbon source, at a level of 6.4 g/l and organisms of the HRI strains of *B. stearothermophilus* (NCIB 11407, NCIB 11408, NCIB 11409, NCIB 11410, NCIB 11411, NCIB 11412, and NCIB 11413) are grown in this medium in batch and continuous culture modes.

Batch culture

An inoculum of .002 g (dry weight) of organisms of one HRI strain of *B. stearothermophilus* is added to 1.5 l of batch culture of the above medium in a 2 l. culture container, and grown with aeration at 65°C with a doubling time of 80 minutes. The yield of organisms obtained is about 0.2 g dry mass cells per litre of medium and the optimum time in the growth curve for cell harvesting to yield maximum specific activity for the enzyme is found to be in the late log phase of the growth cycle. A typical enzyme activity obtained is 20 μ moles o-nitrophenyl-β-galacto-pyranoside per minute per mg dry weight of cells.

Continuous culture

Similarly as for batch culture 0.003 g (dry weight) of the HRI strain of *B. stearothermophilus* is inoculated in to 0.75 l of the same medium contained in a 1 l. glass continuous culture container and grown at 65°C. It is found that when the organism is grown under conditions of oxygen limitation the specific activity of the enzyme is decreased, and that enzyme yield increases with dilution rate up to an

optimum of 0.1 under oxygen limiting conditions. A yield of 0.4 mg dry weight of cells is obtained per litre of medium.

Similar culture medium and growth conditions, as above, are used to grow strains of *B. stearothermophilus* as an initial indicator of their suitability for derivation of the enzyme, enzyme compositions and enzymatically active whole cell preparations of the invention. Growth in such culture media under such conditions e.g. temperatures around 65°C, is *prima facie* evidence of the suitability of the organism though ultimate suitability is determined from the thermal stability characteristics of the β-galactosidase isolated from the organism.

Example 2

Preparation of enzyme extracts and purified enzyme

A quantity of 20 g dry weight of washed cells of an HRI strain of *B. stearothermophilus,* as prepared in Example 1, is resuspended in .15 l of buffer containing 0.15 M potassium chloride and treated with lysozyme by the method of Griffith and Sundaram (J. Bacteriol 1973, *116,* 1160) to provide a crude cell extract. The cell-free extract thus obtained is made 25% saturated with respect to ammonium sulphate, the precipitate formed is removed and more ammonium sulphate is added to the supernatant to make a 70% saturated solution. The precipitate thus formed is collected by centrifugation, dissolved in a small quantity of buffer and applied to a Sepharose 6B column (2.6 x 88 cms). The protein is eluted from the column with 50 mM sodium phosphate buffer, pH 7, and the fractions containing β-galactosidase activity are identified by assay and recovered. The pooled enzyme containing fractions are applied to a DEAE-Sepharose CL-6B column (1.6 x 48 cms) and the protein is eluted with a linear gradient of 0—2M sodium chloride. The β-galactosidase containing fractions are pooled and dialysed overnight against 50mM sodium phosphate buffer, pH 7.

An enzyme solution is produced having a specific activity of about 7,500 μ/g, where one unit hydrolyses 1 μ mole of ONPG per minute at pH 7.0 and 65°C.

Example 3

Production of whole cell suspension and immobilised whole suspension cell preparations

A quantity of 20 g dry weight of cells of an HRI strain of *B. stearothermophilus,* as prepared in Example 1, is suspended in .15 l. of 50mM sodium phosphate buffer, pH 7, and a 2:1 solution of toluene-acetone is added at the rate of 1 ml per 10 ml of suspension. The cells are shaken at room temperature for about 5 minutes, and then harvested by centrifugation at 6000 g for 20 minutes in an MSE "Hi Spin 21" centrifuge prior to washing with buffer. The enzymatically active whole cell product obtained is resuspended in buffer to give a cell density of 20.2 mg dry wt/ml. The relation of Harris and Kornberg (Proc. Roy. Soc. Lond. B. 1972, *182,* 159) is used to determine the cell density, 0.33 mg. dry wt. of *B. stearothermophilus* corresponding to an absorbance of 1.0 at 680 n.m.

Immobilised cells

Washed cells which have been toluene treated, as above, are resuspended in an equal volume of 50 mM sodium phosphate buffer, pH 7.0, containing acrylamide (200 g/l) and N,N'-methylenebisacrylamide (20 g/l). Ammonium persulphate (2 g/l) and TEMED (2 ml/l) are added to this suspension which is then allowed to gel, and the gel is disrupted by forcing through a wire mesh, as described by Hjerten (Arch. Biochem. Biophys. Suppl. 1962, *1,* 147). The disrupted gel is freeze-dried to provide a stock of enzymatically active immobilised whole cell preparation.

Example 4

Assay and characterisation of β-galactosidase

The purified enzyme and enzymatically active whole cell preparations, as prepared in Examples 2 and 3, are assayed under various conditions. The results obtained are characteristic of the HRI enzyme, though, without prejudice, it is believed that these results are representative of the characteristics of other novel β-galactosidases of the invention. The general techniques used for assay are as follows:—

Use of o-nitrophenyl-β-D-galactopyranoside (ONPG) as substrate

The assay used is based on that of Lederberg (J. Bacteriol. 1950, *60,* 381), using an assay mixture comprising 2.8 ml of 50 mM sodium phosphate, pH 7.0, and 0.1 ml of 68 mM ONPG. 0.1 ml aliquots of both purified enzyme and toluene-treated cell suspension, and 10 mg. amounts in 2.9 ml of buffer of freeze dried immobilised whole cell preparation are used for the assays. Assays are carried out at incubation temperature of 65°C and after the appropriate time interval the reactions are halted by addition of 3 ml aliquots of 0.5 M sodium bicarbonate to each reaction mixture and immediate cooling in an ice bath. The absorbance of the resultant solutions is measured at 410 nm using a Perkin-Elmer 124 double beam spectrophotometer. In the case of whole cell suspensions the assay tubes are centrifuged and in the case of the immobilised cells the mixtures are filtered, to remove cells and polyacrylamide gel respectively prior to measurement of the absorbance.

During experiments to determine the pH optimum of the enzyme, the usual sodium phosphate

buffer was replaced by McIlvaines citric acid-phosphate buffer (Elving, Markowitz and Rosenthal, Analyt. Chem. 1956, *28,* 1179) at appropriate pHs.

Use of lactose as substrate

The assay mixture used comprises 0.9 ml of 50 mM sodium phosphate buffer, pH 7.0, 4 ml of lactose (4 g in 100 ml of phosphate buffer), to which 0.1 ml of purified enzyme or cell suspension or 10 mg of gel entrapped cell preparation is added. The reaction mixtures are incubated for 15 minutes at 65°C, reactions are halted by addition of 4.2% perchloric acid and the solutions are centrifuged. Aliquots of the supernatant solutions are assayed for glucose or galactose.

Glucose is estimated by a modification of the method of Trinder (Ann. Clin. Biochem. 1969 *6*, 24). 1 ml aliquots of the supernatant solutions are assayed by addition to a mixture comprising 1 ml of 0.2% phenol and 3 ml of colour reagent (75 ml 4% w/v) disodium hydrogen orthophosphate, 225 ml of water, 6 mg of glucose oxidase (Boehringer Corp. Ltd.), 0.15 ml peroxidase (Boehringer Corp. Ltd.) 100 mg of 4-aminophenazone and 300 mg of sodium azide) followed by incubation at 37°C for 10 minutes and absorbance measurement at 515 nm.

Galactose is estimated as follows: a sample (e.g. 0.2 ml) containing not more than 200 μg/ml of galactose is mixed with 2.5 ml of buffer (4.8 g Na_2HPO_4, 0.86 g NaH_2PO_4 and 0.2 g $MgSO_4$ $7H_2O$ per 200 ml of H_2O) and 0.1 ml of 10 mg/ml of NAD solution and the absorbance is measured at 340 nm. 0.02 ml of galactose dehydrogenase (Boehringer Corp. Ltd.) is added, the solutions incubated at 37°C for 30 minutes and the absorbance again measured at 340 nm.

Thermostability measurements

To determine the thermostability of the enzyme preparations, 0.5 ml aliquots of purified enzyme (2.7 mg/ml of protein), or cell suspension, or 10 mg amounts in 2.9 ml of 50 mM sodium buffer of the freeze-dried gel immobilised preparation, are heated in sealed tubes at appropriate temperatures for the requisite periods of time. After such heat treatment the residual β-galactosidase activity is assayed using ONPG as substrate. .1 ml aliquots of purified enzyme or cell suspension are removed from the tubes and assayed for enzyme activity. In the case of immobilised whole cells the residual β-galactosidase activity is assayed by adding ONPG (0.1 ml) to the reaction mixtures and following the usual procedure detailed previously for use of ONPG as substrate. This procedure for thermostability measurement may be used to determine whether enzyme from strains of *B. stearothermophilus* are novel enzymes according to the present invention.

Lactose determination

The lactose concentration of milk and milk products is determined by the method of Nickerson et al (J. Dairy Sci. 1976, *59,* 386).

(a) Effect of temperature on enzyme activity

The effect of temperature on the relative activity of β-galactosidase in whole cell suspension preparation (curve 1), entrapped whole cell preparation (curve 2) and purified enzyme extract (curve 3) is shown in Figure 1, the temperature for optimum activity being 75°, 65° and 60°C respectively. In Figure 1 the activity is given in terms of μ moles of nitrophenol formed per minute per mg of protein or per mg dry weight of cells and the differing scales used are marked in accordance with their corresponding curves.

From the data obtained the activation energies are determined and found to be closely similar for the purified enzyme and the whole cell preparation (69.2 k cal/mol. and 61.8 k cal/mol respectively), though somewhat higher (155.7 k cal/mol) for the entrapped whole cell preparation. This latter observation is believed to be caused by the effect of diffusion processes within the gel matrix.

(b) Effect of pH on enzyme activity

The pH-activity profiles for the β-galactosidase activities of whole cell suspension preparation (curve 1), entrapped whole cell preparation (curve 2) and purified enzyme extract (curve 3) are shown in Figure 2. The pH optima are 6.2, 6.6 and 6.0 respectively, though all three sources of enzyme show activity over a wide range of pH. The units and arrangement of Figure 2 are similar to those of Figure 1.

(c) Stability of enzyme on prolonged incubation

The results obtained for enzyme activity after incubation at various temperatures is given below in Table 2.

TABLE 2

Thermostability of β-galactosidase from the HRI strains of B. stearothermophilus using whole cells, purified enzyme and immobilised whole cells as enzyme sources

Temperature °C	Half-life (Mins) of β-galactosidase Activity		
	Whole cells	Purified Enzyme	Immobilized Whole cells
55	635	No inactivation after 90 mins	800
60	355	450	530
65	190	85	230
70	125	20	25

The results obtained evidence the extremely good thermal stability of the β-galactosidase in all three sources at temperatures of 55 and 60°C. At higher temperatures the enzyme activity of the whole cell suspension preparation becomes markedly more stable than that of the purified enzyme.

(d) Michaelis constant for ONPG and lactose

The Michaelis constants (Km) with ONPG as substrate for the β-galactosidase activity of whole cell suspension, purified enzyme and entrapped whole cell preparation are found to be 3.8×10^{-4}M, 4.3×10^{-4}M and 2.6×10^{-4}M respectively.

With lactose as substrate, however, the Kms for the β-galactosidase of whole cell suspension and entrapped whole cell preparation are found to be 1.18×10^{-2} M and 9.52×10^{-3}M respectively whereas that for purified enzyme extract is found to be 2.06×10^{-3}M.

(e) Inhibition of enzyme by glucose and galactose

Addition of either glucose or galactose to the assay mixtures inhibits enzyme activity from all sources with both ONPG and lactose as substrate, galactose inhibition being the more marked. For example, the inhibition constant (Ki) for galactose with lactose as substrate using whole cell suspension is 2.3×10^{-2}M, for purified enzyme 2.0×10^{-2}M and for entrapped whole cell preparation 2.6×10^{-2}M. The Ki's for glucose, with lactose as substrate are much higher, being 2.2×10^{-1}M for whole cell suspension, 1.3×10^{-1}M for purified enzyme and 2.6×10^{-1} for entrapped whole cell preparation.

(f) Effect of divalent cations on enzyme activity

The effect on enzyme activity of adding certain divalent cations, cysteine and protein (in the form of bovine serum albumin) is shown in Table 3 below. Enzyme activity is determined using ONPG as substrate, all additions being made to a final concentration of 10mM with the exception of bovine serum albumin which is added to a final concentration of 1.5 mg/ml.

TABLE 3

Effect of certain divalent cations, cysteine and BSA on β-galactosidase activity using whole cell suspension, purified enzyme, and immobilised whole cell preparation of B. stearothermophilus HRI

Addition	Whole Cells		Purified Enzyme		Immobilised Whole Cells	
	Specific Activity mole/min/ mg cells	% Activity	Specific Activity mole/min/ mg. Prot.	% Activity	Specific Activity mole/min/ mg/Gel	% Activity
None	0.427	100	7.002	100	0.032	100
$CaCl_2$	0.428	99.8	6.690	95.5	0.032	100
$MgCl_2$	0.512	119.9	11.114	158.7	0.041	128.1
$MgSO_4$	0.512	119.9	11.110	158.7	0.042	131.3
$FeSO_4$	0.519	121.5	11.556	165.0	0.038	118.8
$CuSO_4$	0.062	14.5	0.548	7.8	0.001	3.1
Cysteine	0.443	103.7	6.866	98.1	0.035	109.4
Bovine Serum Albumin	0.436	102.1	7.804	111.5	0.032	100

Mg^{2+} and Fe^{2+} act as activators for all enzyme sources, Cu^{2+} is a powerful inhibitor, cysteine produces little or no effect and there is slight activation of the purified enzyme in the presence of protein. In all cases where significant activation is achieved the effect is greatest for the purified enzyme.

(g) Effect of β-galactosidase on a range of substrates

The β-galactosidase of HRI strains of *B. stearothermophilus* is found to be active for hydrolysing lactose in whole milk, skim milk, whey, demineralised whey and permeate from whey after ultrafiltration.

With reference to Figure 3, enzyme from all three sources (whole cell suspension — curve 1, immobilised whole cell preparation — curve 2 and purified enzyme — curve 3) is active in hydrolysing the lactose of demineralised whey. Figure 3 is given in terms of mg of glucose produced (y axis) against time in minutes (x axis). Thus the conversion of lactose after 30 minutes at 65°C is about 7.5% for whole cell suspension, 40% for purified enzyme and 5% for immobilised whole cell preparation.

Also, the action of purified enzyme on the hydrolysis of lactose in whole milk is shown in Figure 4, which is given in terms of lactose content (y axis; mg/ml) of the milk against time (x axis; hours). After 4 hours at 65°C about 30% hydrolysis is achieved by only 14 units of enzyme in 20 ml of whole milk.

Example 5

Production assay and characterisation of ion-exchange support material — immobilised whole cell preparation

Cells of the HRI strain of *B. stearothermophilus* are grown under continuous culture conditions as described in Example 1. The cells are then immobilised by attachment to DEAE-cellulose (Vistec D.1. cellulose media. S. grade Koch-Light Laboratories Limited), amino-ethyl cellulose (Sigma Chemical Company Limited), DEAE-Sephadex A50 (Pharmacia) and DEAE-Sepharose CL6B (Pharmacia), the same immobilisation procedure being employed in each case. The procedure used is described in detail below for DEAE-cellulose.

Dry DEAE-cellulose is stirred into 15 volumes of 0.5 N HCl, allowed to stand for 30 minutes, and the supernatant removed by suction filtration using a Buchner funnel. The cellulose is then washed with distilled water until the pH of the washings is between 4.0 and 8.0. The washed media is stirred into 15 volumes of 0.5 N NaOH and, after 30 minutes, the alkali is removed and the cellulose washed with

water until the washings are at neutral pH. The washed DEAE-cellulose is resuspended in 50 mM sodium phosphate buffer, pH 7.0, and glutaraldehyde (25% w/v aqueous solution) added to give a final concentration of 1% w/v. After stirring for 2 hours at room temperature, the cellulose derivative is recovered by filtration, and the excess glutaraldehyde removed by washing with the sodium phosphate buffer (50 mM, pH 7). The DEAE-cellulose is resuspended in buffer and an equal volume of a suspension in the same buffer of cells of *B. stearothermophilus* (40 mg dry mass per ml) as prepared above, is added. The suspension is stirred for a further 2 hours at room temperature and the immobilised cells are recovered by filtration. The immobilised cells are washed with sodium phosphate buffer containing 0.5 M NaCl to remove non-covalently bound cells, followed by washing with buffer containing no added NaCl.

Comparison of beta-galactosidase activity of B. stearothermophilus cells attached to various ion-exchange supports

Four jacketed chromatography columns (A. Gallenkamp and Company Limited) are filled with cells immobilised as described above, one column for each of the different kinds of ion-exchange support material used. A 5% w/v lactose solution in 50 mM phosphate buffer, pH 7.0, is eluted upwards through the columns. The columns are thermostatted at 60°C, and samples of the eluate are assayed for glucose/galactose content at regular intervals.

The results obtained are given in the accompanying diagram, Figure 5, the extent of hydrolysis being expressed in terms of mg of glucose/galactose formed per hour per ml. of column volume. The results for the DEAE-cellulose, DEAE-Sephadex A50, DEAE-Sepharose CL6B and amino-ethyl cellulose supports are given by lines 1, 2, 3 and 4 respectively. By far the greatest activity is observed with the column packed with cells immobilised on DEAE-cellulose. This result, however, does not reflect the total ion exchange capacity of the gels, and although amino-ethyl cellulose has the lowest capacity (0.3 meq. per g) both DEAE-Sephadex A50 (3.5 m eq. per g) and DEAE-Sepharose (approximately 2.2 m eq. per g at pH 7) both have higher capacities than DEAE-cellulose (1 m eq. per g).

In view of the outstanding properties of glutaraldehyde-treated DEAE-cellulose immobilised cells as compared with cells immobilised on other supports further investigation and characterisation is restricted to this material. First of all, however, the binding capacity of glutaraldehyde-treated DEAE-cellulose for whole cells of *B. stearothermophilus* and the activity of the beta-galactosidase of these immobilised cells is investigated. The amount of cells bound to the glutaraldehyde-treated DEAE-cellulose is estimated by determining the dry mass of cells remaining after attachment to the matrix, and comparing that with the dry mass of the original cell suspension, the difference being taken as the dry mass of cells which bind to the cellulose material. These investigations show that the weight of cells bound appears to vary with treatment, but on average between 2 and 4 mg dry mass of cells are bound per g of DEAE-cellulose. This is lower than is found for DEAE-cellulose which has not been treated with glutaraldehyde (about 4—8 mg dry mass of cells per g of support) suggesting that not all the available reactive sites of the DEAE-cellulose are occupied by glutaraldehyde molecules.

The beta-galactosidase activity of cells immobilised on DEAE-cellulose is assayed using ONPG as substrate under standard conditions, as in Example 4, and that for glutaraldehyde-treated DEAE-cellulose support appears to be higher than the activity remaining in cells absorbed on to untreated DEAE-cellulose (about 7.7 and 3.5 units per milligram dry mass of cells immobilised respectively). The beta-galactosidase activity of the glutaraldehyde-treated gel bed is approximately 22 units per g of support.

Effect of flow rate of substrate through columns

A 5% w/v lactose solution in 50 mM phosphate buffer, pH 7.0, is eluted at various flow rates through columns maintained at 60°C containing glutaraldehyde-treated DEAE-cellulose immobilised cells (bed volumes 66.5 ml). Samples are collected and the lactose concentration of the eluate is determined, and the results are given in the accompanying diagram, Figure 6. The results show that by varying the flow rate, greater than 80% hydrolysis of the lactose solution can be achieved.

The effect of pH on the activity of Beta-galactosidase of immobilised cells

Columns are packed with cells immobilised on glutaraldehyde-treated DEAE-cellulose (bed volume 73 ml) and 5% w/v lactose solution in sodium phosphate buffers of ionic strength 0.1 and various pH's are eluted through the beds at a flow rate of approximately 19 ml per hour. Eluate samples are removed at regular intervals over several days and assayed to determine the lactose concentratoin present. Half lives are estimated by regression analysis of graphs of lactose converted per hour against time. The results obtained are given below in Table 4.

TABLE 4

Apparent half lives of β-galactosidase activity of immobilised cells at different pH's

pH	Half-life (Days)
5.5	1.5*
6.0	4.5
6.6	10
7.0	16.5
7.4	33

* at this pH the lactose solution was buffered with McIlvaines citric acid-phosphate buffer.

Also the results obtained for β-galactosidase activity at the various pH's are given in the accompanying diagram, Figure 7. Thus the thermal stability of the product appears to increase with increasing pH though the optimum pH for activity appears to be about 6.4.

Effect of temperature on the beta-galactosidase activity of immobilised cells

A 5% w/v solution of lactose in 50 mM phosphate buffer, pH 7.0, is eluted through columns containing glutaraldehyde-treated DEAE-cellulose immobilised cells (bed volume 58.5 ml). The columns are maintained at different temperatures and activity of the enzyme is determined by assaying the glucose and galactose concentrations in the eluate stream over a period of up to 4 days. The results obtained are given in the accompanying diagram, Figure 8(a) and an Arrhenius plot of the beta-galactosidase activity is calculated from these results and is given in Figure 8(b).

The half lives of beta-galactosidase activity of immobilised cells are also determined and the results are given in Table 5 below. Half lives are estimated by regression analysis of the results given in Figure 8(a).

TABLE 5

Apparent half-lives of β-galactosidase activity of immobilised cells at different temperatures

Temperature (°C)	Half life (Days)
45	68.5
50	45
55	19
60	15
65	8.5
70	3.5

Effect of ionic strength on the beta-galactosidase activity of immobilised cells

Lactose solutions (5% w/v) are prepared in phosphate buffers (pH 7.0) of differing ionic strengths, and the activity half lives of the immobilised cells are determined, the results being given in the accompanying diagram Figure 9. 5% w/v solutions of lactose in sodium phosphate buffers, pH 7.0 and ionic strengths of 0.1 (line 1); 0.5 (line 2); and 0.85 (line 3); are eluted through columns containing glutaraldehyde-treated DEAE-cellulose immobilised cells (bed volume 66.5 ml) at flow rates of approximately 20 ml per hour. The column temperatures are maintained at 60°C and samples of the eluate are removed at intervals and assayed for lactose content. The activity of the enzyme is expressed as the amount of lactose hydrolysed per hour, and half lives are determined by regression analysis assuming linear decay of the enzyme activity with time.

Activity of the beta-galactosidase of immobilised cells on various substrates

Pure lactose, demineralised whey and skimmed milk are used as lactose sources for the glutaraldehyde-treated DEAE-cellulose immobilised cells and the results obtained are given in the accompanying diagram, Figure 10. A 5% solution of lactose in 50 mM phosphate buffer, pH 7.0 (line 1); a solution of demineralised, ultrafiltered whey containing 48.95 mg per ml of lactose and at pH 6.55 (line 2); and skim milk containing 48.50 mg per ml of lactose and at pH 6.86 (line 3), are eluted through a column maintained at 60°C containing glutaraldehyde-treated DEAE-cellulose immobilised cells (bed volume 41 ml). The flow rates are 28.3, 29.5 and 29.5 ml per hour respectively for the three substrates.

The amount of glucose/galactose formed in samples of eluate is determined and the percentage hydrolysis calculated.

Claims

1. A thermally stable β-galactosidase enzyme derived from a strain of *Bacillus stearothermophilus,* characterised in that it has a thermal stability such that when in purified form it has an activity half life of at least 1½ hours at 55°C, at least 1 hour at 60°C and at least 10 minutes at 65°C, as measured using ONPG as substrate, and in that it is derived from a strain of *B. stearothermophilus* identified as NCIB 11407, NCIB 11408, NCIB 11409, NCIB 11410, NCIB 11411, NCIB 11412 or NCIB 11413 or a variant or mutant thereof.

2. A β-galactosidase according to Claim 1, in purified form.

3. An immobilised β-galactosidase composition comprising a beta-galactosidase enzyme according to Claim 1, in which the enzyme is immobilised with a suitable solid phase material.

4. An enzymically active whole cell preparation of bacteria of species *Bacillus stearothermophilus* which contain a β-galactosidase according to Claim 1.

5. An immobilised enzymically active whole cell preparation according to Claim 4, in which the cells are immobilised on or with an ion-exchange support material.

6. An immobilised enzymically active whole cell preparation according to Claim 5, in which the ion-exchange support comprises DEAE-cellulose or like material.

7. A process for the production of a product according to any of the preceding claims, comprising culturing in a medium containing appropriate nutrients a micro-organism of species *Bacillus stearothermophilus* identified as NCIB 11407, NCIB 11408, NCIB 11409, NCIB 11410, NCIB 11411, NCIB 11412 and NCIB 11413 or a variant or mutant thereof, and which contains an enzyme according to Claim 1.

8. A process according to Claim 7, in which the medium contains lactose as the sole carbon source and cultivation is carried out at a temperature of at least 60°C.

9. A process according to Claim 7 or 8, in which subsequent to cultivation, enzyme is extracted from the bacteria.

10. A process according to Claim 7 or 8, in which, subsequent to cultivation, the bacteria are treated to render them permeable.

11. A process according to any of Claims 7—10, in which enzyme extract or whole cells are immobilised on or with a suitable solid phase material.

12. A process according to Claim 11, in which whole cells are immobilised in a gel matrix.

13. A process according to Claim 11, in which enzyme extract or whole cells are immobilised with an ion-exchange support material, such as DEAE-cellulose or like material.

14. A process according to Claim 13, in which the ion exchange material is treated with glutaraldehyde prior to immobilisation of enzyme extract or whole cells so as to covalently attach said extract or said cells to the ion-exchange material.

15. A process for the hydrolysis of lactose to a product comprising glucose and galactose, in which lactose is contacted with a beta-galactosidase enzyme, immobilised beta-galactosidase composition or enzymically active whole cell preparation according to any of Claims 1—6.

16. A process according to Claim 15 which is carried out at a temperature of at least 55°C, preferably at least 60°C, especially about 65°C.

17. A process according to Claim 15 or 16 in which the source of lactose comprises whey or other lactose-containing milk product.

18. A micro-organism comprising a bacterium of a strain of *Bacillus stearothermophilus* characterised in that the strain is identified as NCIB 11407, NCIB 11408, NCIB 11409, NCIB 11410, NCIB 11411, NCIB 11412 or NCIB 11413 or a variant or mutant thereof.

Patentansprüche

1. Thermisch stabiles β-Galactosidase-Enzym aus einem Stamm von Bazillus stearothermophilus, dadurch gekennzeichnet, daß es eine solche thermische Stabilität aufweist, daß bei Vorliegen in gereinigter Form seine Aktivitäts-Halbwertszeit mindestens 1½ h bei 55°C, mindestens 1 h bei 60°C und mindestens 10 min bei 65°C beträgt bei der Bestimmung unter Verwendung von ONPG als Substrat, und daß es von einem Stamm von B. stearothermophilus, identifiziert als NCIB 11407, NCIB 11408, NCIB 11409, NCIB 11410, NCIB 11411, NCIB 11412 oder NCIB 11413, oder einer Variante oder Mutante derselben stammt.

2. β-Galactosidase nach Anspruch 1 in gereinigter Form.

3. Immobilisierte β-Galactosidas-Zusammensetzung, gekennzeichnet durch ein β-Galactosidase-Enzym nach Anspruch 1, das mit einem geeigneten Festphasenmaterial immobilisiert ist.

4. Enzymatisch aktives Ganzzellenpräparat von Bakterien der Spezies Bazillus stearothermophilus, dadurch gekennzeichnet, daß es eine β-Galactosidas nach Anspruch 1 enthält.

5. Immobilisiertes, enzymatisch aktives Ganzzellenpräparat nach Anspruch 4, dadurch gekennzeichnet, daß die Zellen auf einem oder mit einem Ionenaustauscher-Trägermaterial immobilisiert sind.

6. Immobilisiertes, enzymatisch aktives Ganzzellenpräparat nach Anspruch 5, dadurch gekennzeichnet, daß das Ionen-austauscher-Trägermaterial DEAE-Cellulose oder dergleichen Material aufweist.

7. Verfahren zur Herstellung eines Produktes nach Ansprüchen 1 bis 6, dadurch gekennzeichnet, daß in einem geeignete Nährstoffe enthaltenden Medium ein Mikroorganismus der Spezies Bazillus stearothermophilus, identifiziert als NCIB 11407, NCIB 11408, NCIB 11409, NCIB 11410, NCIB 11411, NCIB 11412, NCIB 11413 oder eine Variante oder Mutante derselben, die ein Enzym nach Anspruch 1 enthält, kultiviert, wird.

8. Verfahren nach Anspruch 7, dadurch gekennzeichnet, daß das Medium Lactose als einzige Kohlenstoffquelle enthält, und die Kultivierung bei einer Temperatur von mindestens 60°C durchgeführt wird.

9. Verfahren nach Ansprüchen 7 oder 8, dadurch gekennzeichnet, daß das Enzym nach der Kultivierung von den Bakterien extrahiert wird.

10. Verfahren nach Ansprüchen 7 oder 8, dadurch gekennzeichnet, daß die Bakterien nach der Kultivierung behandelt werden, um sie permeabel zu machen.

11. Verfahren nach Ansprüchen 7 bis 10, dadurch gekennzeichnet, daß Enzymextrakt oder ganze Zellen auf oder mit einem geeigneten Feststoffmaterial immobilisiert werden.

12. Verfahren nach Anspruch 11, dadurch gekennzeichnet, daß ganze Zellen in einer Gelmatrix immobilisiert werden.

13. Verfahren nach Anspruch 11, dadurch gekennzeichnet, daß der Enzymextrakt oder die ganzen Zellen mit einem Ionen-austauscher-Trägermaterial wie DEAE-Cellulose oder dgl. immobilisiert werden.

14. Verfahren nach Anspruch 13, dadurch gekennzeichnet, daß das Ionenaustauscher-Trägermaterial vor der Immobilisierung des Enzymextrakts oder der ganzen Zellen mit Glutaraldehyd behandelt wird zur kovalenten Bindung des Extrakts oder der Zellen an das Ionenaustauscher-Material.

15. Verfahren zur Hydrolyse von Lactose zu einem glucose- und galactose haltigen Produkt, dadurch gekennzeichnet, daß die Lactose mit einem β-Galactosidase Enzym, einer immobilisierten β-Galactosidase-Zusammensetzung oder einem enzymatisch aktiven Ganzzellenpräparat nach Ansprüchen 1 bis 6 in Kontakt gebracht wird.

16. Verfahren nach Anspruch 15, dadurch gekennzeichnet, daß bei einer Temperatur von mindestens 55°C, vorzugsweise von mindestens 60°C, insbesondere von etwa 65°C gearbeitet wird.

17. Verfahren nach Ansprüchen 15 oder 16, dadurch gekennzeichnet, daß als Lactosequelle Molken oder ein anderes lactosehaltiges Milchprodukt eingesetzt wird.

18. Mikroorganismus mit einem Gehalt an einem Bakterium des Stammes Bazillus stearothermophilus, dadurch gekennzeichnet, daß der Stamm identifiziert ist als NCIB 11407, NCIB 11408, NCIB 11409, NCIB 11410, NCIB 11411, NCIB 11412 oder NCIB 11413 oder eine Variante oder Mutante derselben ist.

Revendications

1. Bêta-galactosidase thermiquement stable, enzyme produite par une souche de *Bacillus stearothermophilus* et caractérisée en ce qu'elle possède une stabilité thermique telle que, lorsqu'elle est sous forme purifiée, elle présente une période d'activité ou demi-vie d'au moins une heure et demie à 55°C, d'au moins une heure à 60°C, et d'au moins 10 minutes à 65°C, lorsque la mesure est effectuée à l'aide de ONPG comme substrat, et en ce qu'elle provient d'une souche de *B. stearothermophilus* identifiée comme étant NCIB 11407, NCIB 11408, NCIB 11409, NCIB 11410, NCIB 11411, NCIB 11412 ou NCIB 11413, ou d'un variant ou mutant de ce bacille.

2. Bêta-galactosidase selon la revendication 1, sous forme purifiée.

3. Composition de bêta-galactosidase immobilisée, comprenant une enzyme bêta-galactosidase selon la revendication 1, dans laquelle l'enzyme est immobilisée à l'aide d'une matière convenant pour donner une phase solide.

4. Préparation de cellules entières, à activité enzymatique, de bactéries de l'espèce *Bacillus stearothermophilus* qui contiennent une bêta-galactosidase selon la revendication 1.

5. Préparation de cellules entières immobilisées, à activité enzymatique selon la revendication 4, dans laquelle les cellules sont immobilisées dans ou à l'aide d'une matière de support et d'échange d'ions.

6. Préparation de cellules entières immobilisées, à activité enzymatique selon la revendication 5, dans laquelle le support d'échange d'ions comprend de la DEAE-cellulose ou une matière analogue.

7. Procédé pour obtenir un produit selon l'une quelconque des revendications précédentes, comprenant la culture, dans un milieu contenant des matières nutritives appropriées, d'un micro-organisme de l'espèce *Bacillus stearothermophilus* identifié comme étant NCIB 11407, NCIB 11408, NCIN 11409, NCIB 11410, NCIB 11411, NCIB 11412, NCIB 11413, ou un variant ou mutant de ce

bacille, et qui contient une enzyme selon la revendication 1.

8. Procédé selon la revendication 7, dans lequel le milieu contient du lactose comme seule source de carbone et la culture est effectuée à une température d'au moins 60°C.

9. Procédé selon la revendication 7 ou 8, dans lequel, après la culture, on extrait l'enzyme des bactéries.

10. Procédé selon la revendication 7 ou 8, dans lequel, après la culture, on traite les bactéries pour les rendre perméables.

11. Procédé selon l'une quelconque des revendications 7 à 10, dans lequel on immobilise l'extrait d'enzyme ou les cellules complètes sur ou à l'aide d'une matière convenant pour donner une phase solide.

12. Procédé selon la revendication 11, dans lequel on immobilise les cellules entières sur une matrice ou gel d'enrobage.

13. Procédé selon la revendication 11, dans lequel on immobile de l'extrait d'enzyme ou des cellules entières à l'aide d'une matière de support et d'échange d'ions, comme de la DEAE-cellulose ou une matière analogue.

14. Procédé selon la revendication 13, dans lequel on traite la matière d'échange d'ions avec du glutaraldéhyde avant d'immobiliser l'extrait d'enzyme ou les cellules entières, de manière à fixer par covalence cet extrait ou ces cellules sur la matière d'échange d'ions.

15. Procédé pour l'hydrolyse du lactose pour obtenir un produit comprenant du glucose et du galactose, dans lequel on met le lactose en contact avec une enzyme bêta-galactosidase, une composition de bêta-galactosidase immobilisée ou une préparation de cellules entières à activité enzymatique selon l'une quelconque des revendications 1 à 6.

16. Procédé selon la revendication 15, qui est mis en oeuvre à une température d'au moins 55°C, de préférence au moins 60°C, en particulier 65°C environ.

17. Procédé selon la revendication 15 ou 16, dans lequel la source de lactose comprend du lactosérum ou un autre produit laitier contenant du lactose.

18. Micro-organisme comprenant une bactérie d'une souche de *Bacillus stearothermophilus*, caractérisé en ce que la souche est identifiée comme étant NCIB 11407, NCIB 11408, NCIB 11409, NCIB 11410, NCIB 11411, NCIB 11412 ou NCIB 11413, ou un variant ou mutant de ce bacille.

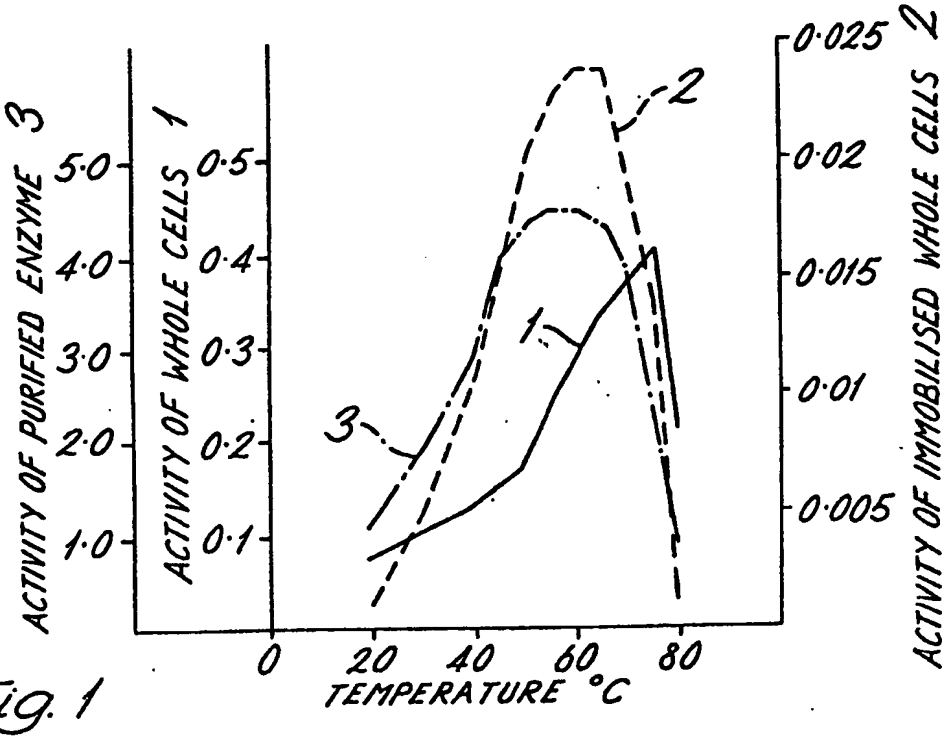
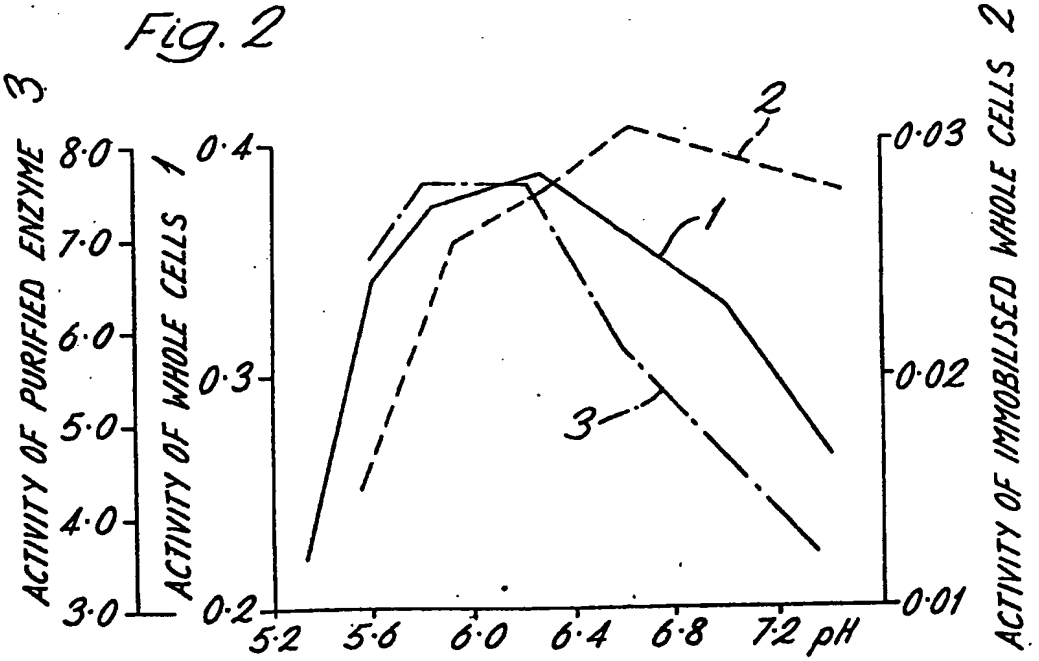

ACTIVITY OF PURIFIED ENZYME 3
5·0
4·0
3·0
2·0
1·0
ACTIVITY OF WHOLE CELLS 1
0·5
0·4
0·3
0·2
0·1
0·025
0·02
0·015
0·01
0·005
ACTIVITY OF IMMOBILISED WHOLE CELLS 2
0
20
40
60
80
TEMPERATURE °C
1
2
3

Fig. 1

Fig. 2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7·0
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ACTIVITY OF WHOLE CELLS 1
0·4
0·3
0·2
0·03
0·02
0·01
ACTIVITY OF IMMOBILISED WHOLE CELLS 2
5·2
5·6
6·0
6·4
6·8
7·2 pH
1
2
3

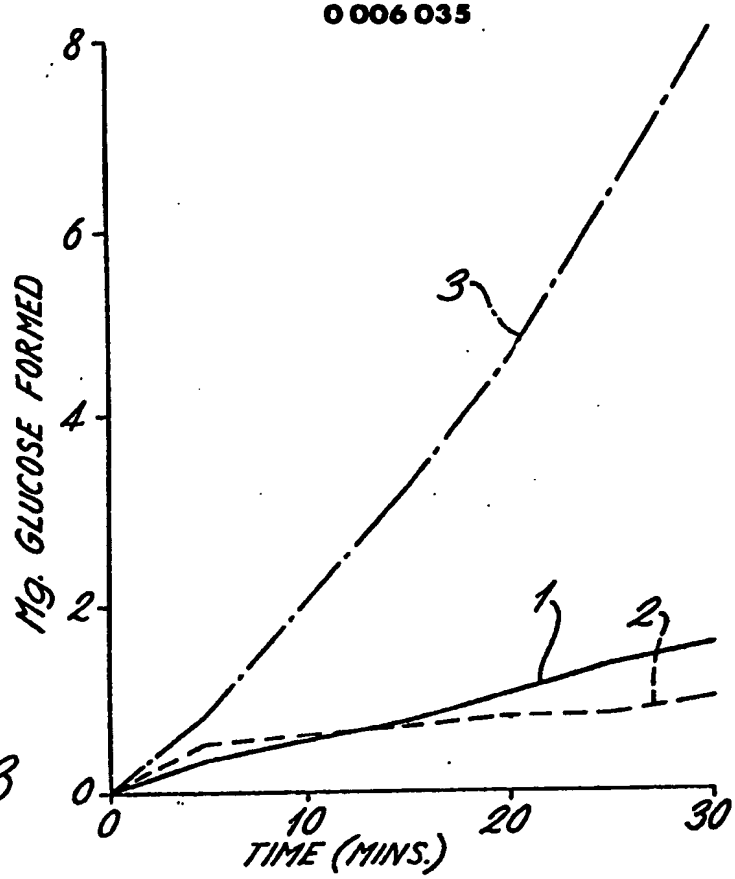
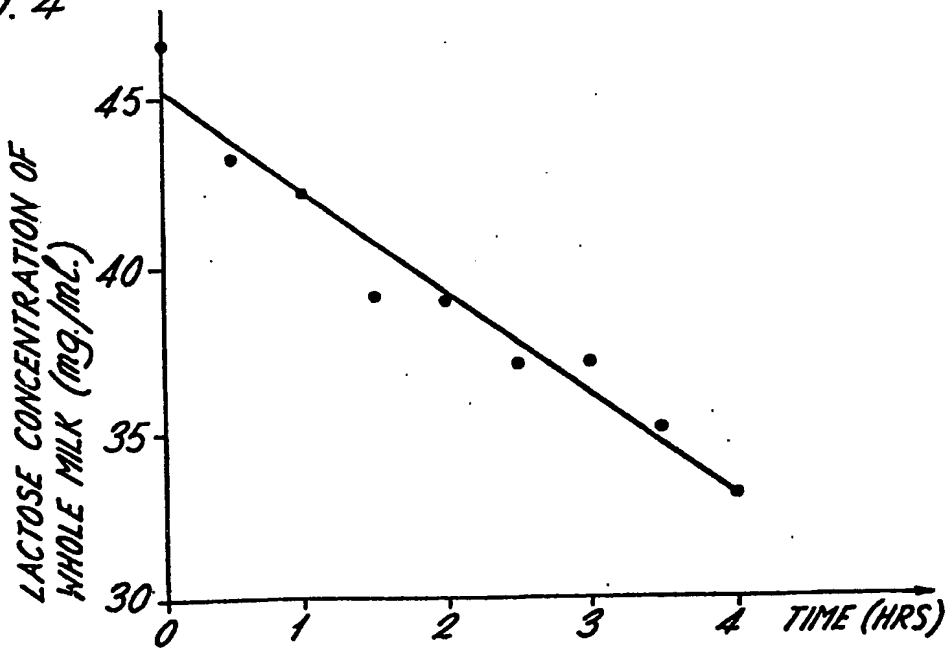

8
6
4
2
0
Mg. GLUCOSE FORMED
3
1
2
0
10
20
30
TIME (MINS.)

Fig. 3

Fig. 4


45
40
35
30
LACTOSE CONCENTRATION OF WHOLE MILK (mg./ml.)
0
1
2
3
4
TIME (HRS)

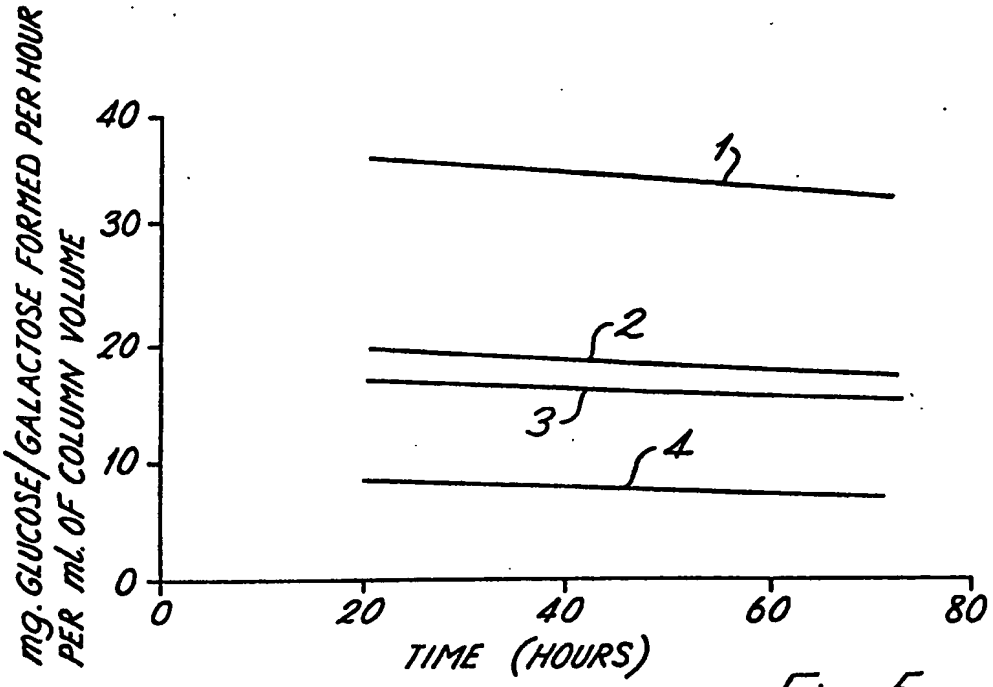


Fig. 5

Fig. 6

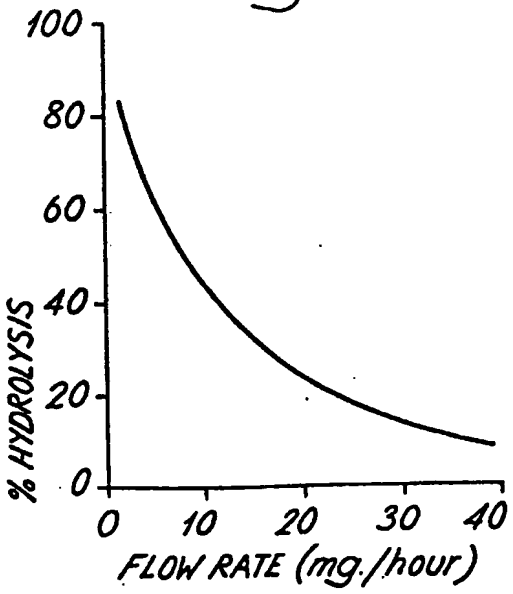
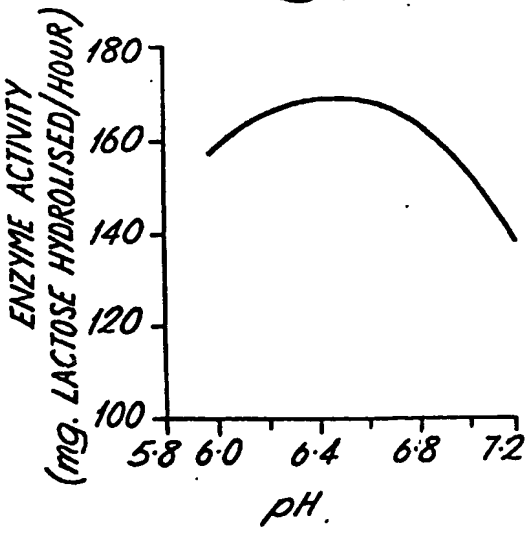


Fig. 7



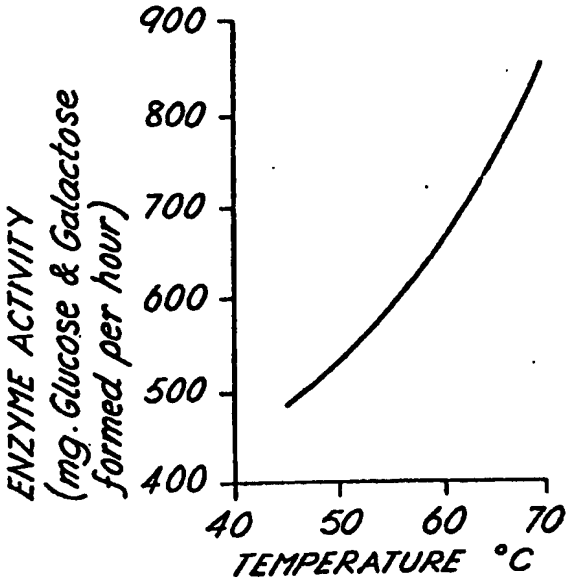
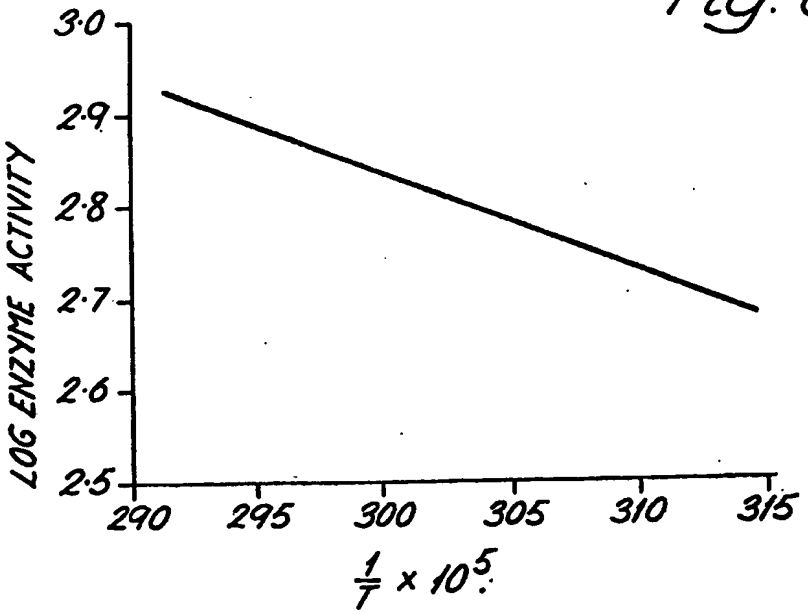

900
800
700
600
500
400
ENZYME ACTIVITY
(mg. Glucose & Galactose
formed per hour)
40
50
60
70
TEMPERATURE °C

Fig. 8a

Fig. 8b


3·0
2·9
2·8
2·7
2·6
2·5
LOG ENZYME ACTIVITY
290
295
300
305
310
315
$\frac{1}{T} \times 10^5$

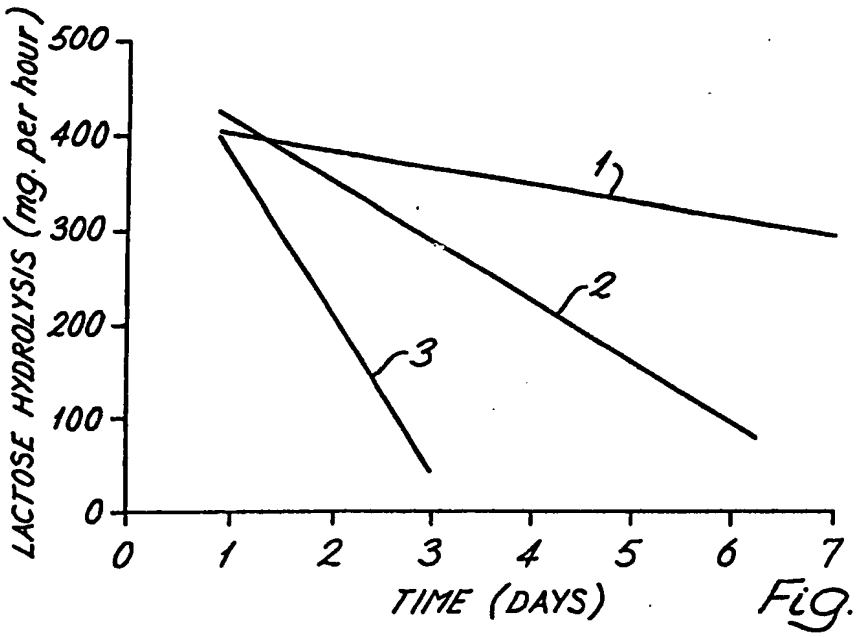

LACTOSE HYDROLYSIS (mg. per hour)
500
400
300
200
100
0
0
1
2
3
4
5
6
7
TIME (DAYS)
1
2
3

Fig. 9

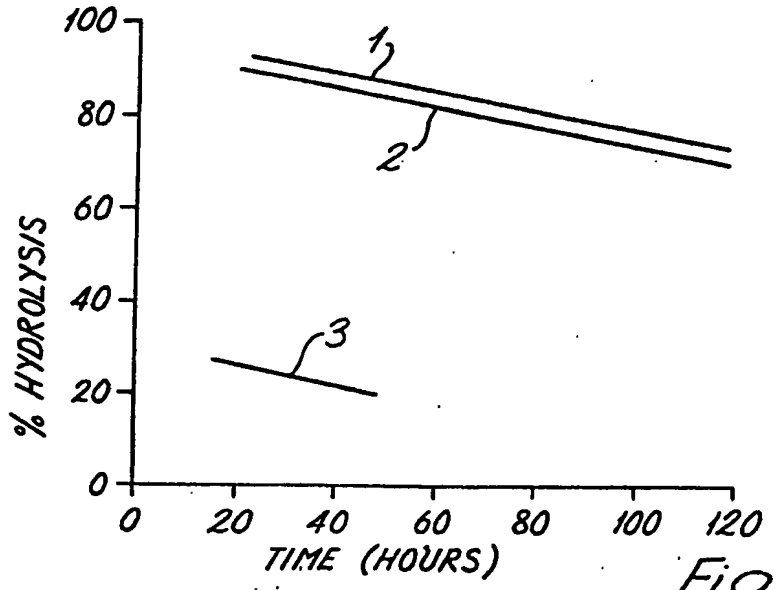

% HYDROLYSIS
100
80
60
40
20
0
0
20
40
60
80
100
120
TIME (HOURS)
1
2
3

Fig. 10